Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note

REG2000-13

Neemix 4.5®

The naturally occurring botanical insecticide Neemix 4.5®, which contains the active ingredient azadirachtin for the control of sawflies in forestry in Canada, has been granted Section 17 temporary registration.

This regulatory note provides a summary of data reviewed and the rationale for the regulatory decision concerning this product.

(publié aussi en français)

October 16, 2000

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Foreword

Health Canada's Pest Management Regulatory Agency (PMRA) has issued a temporary registration for Neemix 4.5®, a naturally occurring botanical insecticide developed by Thermotrilogy Corporation. Neemix 4.5® contains the active ingredient azadirachtin, which is effective against sawflies in forestry.

Thermotrilogy Corporation will be carrying out additional chemistry, toxicological, and efficacy studies as a condition of this temporary registration. Following the review of this new data, the PMRA will publish a proposed registration decision document and request comments from interested parties before proceeding with a final regulatory decision.

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1.0 The active substances, its properties, uses, proposed classification, and labelling

1.1 Identity of the active substance and preparation containing it

Active substance: Azadirachtin

Function: Insecticide

Chemical name (IUPAC): No IUPAC name has been assigned

Chemical name (CAS): dimethyl [2aR-[2*a*",3$,4$(1*a*R*,2*S**,3*aS**,6*aS**,7*S**,7*aS**), 4*a*$,5",7*aS**,=8$(E),10$,10*a*",10*b*$]]-10-(acetyloxy) octahydro-3,5-dihydroxy-4-methyl=8-[(2-methyl-1- oxo-2-butenyl)oxy]-4-[(3*a*,6*a*,7,7*a*)-tetrahydro-6*a*-hydroxy= 7*a*-methyl-2,7-methanofuro[2,3-*b*]oxireno[*e*]oxepin-1*a*(2H)-yl]-1H,7H=naphthol[1,8-*bc*:4,4*a*-*c*N]difuran-5, 10*a*(8H)-dicarboxylate

CAS number: Azadirachtin A 11141-17-6
Azadirachtin B 95507-01-0

Nominal purity of active: 15%

Identity of relevant impurities of toxicological, environmental, or other significance:

> A small amount of aflatoxins may be present in the neem seeds that are the starting material in the manufacture of azadirachtin. The company has established standard operating procedures to minimize the amount of aflatoxins present in its source seeds. Implementing these procedures will insure that the aflatoxin level in the technical product will be a maximum of 80 ppb. Each lot of a technical material will be analysed for the aflatoxin level to insure that it is 80 ppb or less.

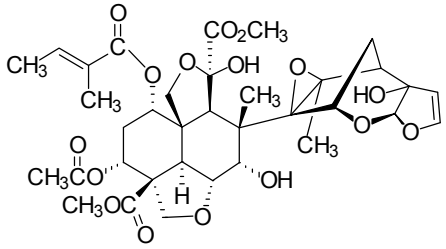
Toxic Substances Management Policy (TSMP) Track 1 substances as identified in Appendix II of Regulatory Directive DIR99-03 *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy* are not expected to be present in the product.

Molecular formula: $C_{35}\ H_{44}\ O_{16}$ (for Azadirachtin A)
$C_{33}\ H_{42}\ O_{14}$ (for Azadirachtin B)

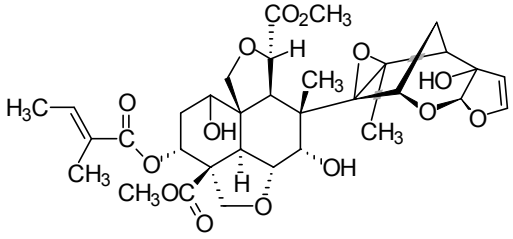
Molecular mass: 720.7 (for Azadirachtin A)
662.7 (for Azadirachtin B)

Structural formula:

Azadirachtin A



Azadirachtin B



1.2 Physical and chemical properties of active substance

Technical product: Azadirachtin

Property	Result
Colour and physical state	Light mustard yellow amorphous solid
Odour	Sulfur
Melting point or range	85–105EC
Boiling point or range	Not applicable
Density	1.2 g/mL at 24EC
Vapour pressure	2.14 mm Hg at 20EC
UV and visible spectrum at 26EC	8_{max} = 220 nm
Water solubility (mg/mL)	2.8×10^{-5} at 10EC 5.0×10^{-5} at 25EC 3.0×10^{-4} at 50EC
Solubility in organic solvents	
acetone	2.0 mg/mL at 10EC
	6.25 mg/mL at 25EC
	9.5 mg/mL at 50EC
ethanol	0.05 mg/mL at 10EC
	0.125 mg/mL at 25EC
	3.75 mg/mL at 50EC
methanol	0.01 mg/mL at 10EC
	0.10 mg/mL at 25EC
	4.25 mg/mL at 50EC
hexane	<200 ppm at 25EC
n-Octanol–water partition coefficient (K_{ow})	12.3 ± 0.2

Property	Result
log K_{ow}	1.09
Dissociation constant	Not applicable, no dissociable moieties
Stability (temperature, metals)	Expected to be stable under conditions of normal use

End-use product: Neemix 4.5®

Property	Result
Colour	Dark reddish brown
Odour	Banana–mint
Physical state	Liquid
Formulation type	Emulsifiable concentrate
Guarantee	4.5%
Container material and description	Plastic 0.5, 1.0, 5.0, and 10.0 L
Density	0.91 g/mL
pH	5.2
Storage stability	Stable when stored for 12 months at room temperature in commercial packaging
Surfactants	Atlox AL-1447

1.3 Details of uses

Neemix 4.5® is intended to be used by air against three sawfly species that are currently causing large scale damage to Canadian forests. It is recommended for control of the balsam fir sawfly (BFS) *Neodiprion abietis* (Harr.), the yellow-headed spruce sawfly (YHSS) *Pikonema alaskensis*, and the pine false webworm (PFW) *Acantholyda erythrocephala* by applying one application of between 20 and 50 g a.i./ha on early instars of larvae.

Balsam fir sawfly is a native species with wide distribution in Canada and the United States. BFS is an increasing problem in balsam fir stands in eastern Canada, most notably in western Newfoundland (for the year 2000, moderate to severe populations are expected in 40 000 ha of forest) and the Cape Breton and Eastern Shore regions of Nova Scotia. Its preferred host is balsam fir, but it may also feed on spruce. The larval stage of BFS feeds on foliage one-year-old and older. One year of feeding damage can cause extensive growth reduction for several years afterwards, making the weakened trees more

susceptible to attack by other organisms. Successive years of defoliation can lead to tree mortality.

Yellow-headed spruce sawfly is a serious pest of plantation and open grown spruce in many regions of North America. In Canada, the problem is particularly pronounced in the Bay of Fundy area and is also a concern in Quebec and Ontario. The young larvae feed only on the new or current year's foliage, but when almost full-grown they will feed on older needles. Persistent infestations will hinder growth development and greatly affect tree appearance, especially of young trees. Trees may even be killed outright after two years or more of severe defoliation, especially when the sawfly outbreak coincides with drought periods.

Pine false webworm is a web-spinning sawfly native to northern Europe and feeds on pines. Initially an occasional pest of young red pine plantations in Ontario, it is now attacking high value, semi-mature and mature red pine plantations, and tree mortality is occurring . It also has become a significant pest of large white pine in Ontario and New York. In Ontario, it is now threatening $40 million worth of red pine plantations.

1.4 Classification and labelling

1.4.1 Azatin 15% Technical

The technical active Azatin 15% Technical is of low acute toxicity via oral, dermal and inhalation routes of exposure, non-irritating to the skin, minimally irritating to the eyes, and not a dermal sensitizer. None of the formulants in Azatin 15% Technical are on the EPA list of Inerts of Toxicological Concern (list 1) or List of Inerts for Priority Testing (list 2).

1.4.2 Neemix 4.5® end-use product

The formulation Neemix 4.5® is of low acute toxicity via oral, dermal, and inhalation routes of exposure, is moderately irritating to eyes, is minimally irritating to skin, and is not a dermal sensitizer. None of the formulants in Neemix 4.5® are on the Environmental Protection Agency (EPA) list of Inerts of Toxicological Concern (list 1) or List of Inerts for Priority Testing (list 2).

2.0 Methods of analysis

2.1 Methods for analysis of the active substance as manufactured

The high-performance liquid chromatography (HPLC) method with UV detection was used for the analysis of the active ingredient and the impurities. The linear range of the detector was sufficiently wide, and the method precision and accuracy were acceptable. The method provided was assessed and fully validated for the active ingredient.

The method linearity and specificity for the impurities was also confirmed. The information on precision and accuracy for the impurities was not provided. However, because of the biological and complex nature of the impurities, the requirement for accuracy and precision of the method has been waived.

2.2 Method for formulation analysis

An HPLC method with UV detection was used for the determination of the active ingredient in this product. The method has satisfactory specificity, linearity, precision, and accuracy and is suitable for use as an enforcement method.

3.0 Impact on human and animal health

3.1 Integrated toxicological summary

Azadirachtin (insect growth regulator) is the active compound in the technical active ingredients Neem Concentrate TGAI and Azatin 15% Technical, both of which contain a neem seed extract from the neem tree *Azadirachta indica* that grows in sections of India, Africa, Indonesia, and South America. Two data packages were submitted by the same registrant to support different uses. Because of deficiencies in both packages and the fact that the source of the two technical actives was the same (the hydrophilic moiety), the PMRA combined the available data from both packages for a more comprehensive review that allowed the establishment of no observed adverse effect levels (NOAELs) and conclusions regarding the potential for adverse health effects.

Neem Concentrate TGAI is of low acute toxicity via the oral and dermal routes of exposure, slightly toxic via the inhalation route of exposure, mildly irritating to eyes, slightly irritating to skin, and not a dermal sensitizer.

Azatin 15% Technical is of low acute toxicity via the oral, dermal, and inhalation routes of exposure, minimally irritating to eyes, non-irritating to skin, and not a dermal sensitizer. The formulation Neemix 4.5® is considered to be of low acute toxicity by the oral, dermal, and inhalation routes of exposure, moderately irritating to eyes, mildly irritating to skin, and not a dermal sensitizer.

Two short-term studies conducted in rats illustrated effects on haematological parameters (decreased mean corpuscular volume(MCV) and mean corpuscular haemoglobin (MCH), suggesting a slight hypochromic and microcytic anemia) at levels greater than 632 mg/kg bw/d. Leukocyte, lymphocyte, monocyte, and reticulocyte numbers were affected at the limit dose of 1000 mg/kg bw/d. The principal target organ was the liver, with increased liver weights and altered clinical chemistry parameters. At the limit dose of 1000 mg/kg bw/d, bile duct proliferation was also observed. The compound also caused effects on kidney, heart, adrenal gland, and ovary weights; however, no histopathological correlates were found for these organs. Gender sensitivity was not clearly evident in rats: the male was more sensitive showing more severe proliferation of

the bile ducts in the portal areas of the liver, whereas females demonstrated increased liver weights and increased gamma glutamyl transpeptidase levels at a lower dose level. The latter incidence may indicate possible hepatobiliary lesions. In the absence of chronic toxicity and carcinogenicity studies, the potential for the compound to cause toxicity following long-term exposure cannot be ruled out.

Although a decrease in adrenal and (or) ovary weights was noted in rats following 90-day dietary exposure, no histopathological correlates were found. However, based on the endocrine mode of action in insects and the absence of a reproductive toxicity study, the potential for the compound to cause endocrine effects cannot be ruled out. No neurological signs of toxicity were observed following dietary or gavage exposure at the limit dose of 1000 mg/kg bw/d.

Neem Concentrate TGAI was not mutagenic in bacterial and mammalian species in vitro and was found to be negative for inducing structural chromosomal aberrations in mice in vivo. Azatin 15% Technical was also not mutagenic in bacterial species. A developmental toxicity study with Neem Concentrate TGAI in rats demonstrated no toxic effects on the dams and no evidence was found of embryo or fetal toxicity or teratogenicity up to the limit dose of 1000 mg/kg bw/d.

Immunotoxicity was demonstrated in a study of Neem Concentrate TGAI treatment via oral gavage in female mice. In this study, body weight decreased by $30% and food consumption was significantly reduced. Severe stress and malnutrition were related to an indirect immunomodulating effect. Although the dose selection may not be appropriate, the observed effects on spleen weight combined with the effects on plaque-forming cell (PFC) assay and the natural killer (NK) cell function confirm that Neem Concentrate TGAI can affect immune responses and that the effects may have clinical significance. None of these effects were observed when mice were dosed with Azatin 15% Technical via the dietary route, up to the highest dose of 1100 mg/kg bw/d. However, Azatin 15% Technical via dietary exposure caused suppression of cytotoxic T-lymphocyte function. In this study, the viability of the splenocytes was not reported, so it is possible that the results seen in the cytotoxic T-lymphocyte function test are associated with decreased viability of splenocytes and are not related to dosing.

Although limited, both data sets indicate potential immunotoxicity effects. Adequate immunotoxicity testing (Tier I) should be performed for Azatin 15% Technical and Neem Concentrate TGAI to support both the forestry use and any uses with potential for subchronic and chronic exposure. The results of Tier I testing will determine a need for Tier II immunotoxicity data.

For the short-term occupational exposure proposed for this forestry application, the lowest observed adverse effect level (LOAEL) based on effects on cytotoxic T-lymphocytes (500 ppm; 112 mg Azatin 15% Technical/kg bw/d) will be used. Other safety factors will be added to full personal protective equipment for workers to ensure that worker exposure is minimized. A full toxicology data package is required before any

expansion of forestry use or other uses involving subchronic and chronic exposure is considered for this product. This is based on the following:

(i) evidence suggesting potential immunosuppression and lack of chronic data in two species to rule out the effect of immunosuppression on tumour formation;

(ii) concern for potential adverse effects on endocrine system; compound has an endocrine mode of action in insects; 90-day rat dietary study demonstrated increases in adrenal and (or) ovary weights; no reproduction study available; and

(iii) literature references indicating that neem oil (hydrophobic fraction of neem seed extract) has been associated with adverse reproductive effects (spermicidal activity, implantation failure; neem oil use as topical contraceptive in humans).

3.2 Determination of acceptable daily intake

Not being established.

3.3 Acute reference dose

Not being established.

3.4 Toxicology end-point selection for occupational and bystander risk assessment

Azatin 15% Technical is of low acute toxicity via oral, dermal, and inhalation routes of exposure, minimally irritating to eyes, non-irritating to skin, and not a dermal sensitizer. The formulation Neemix 4.5® is considered to be of low acute toxicity by the oral, dermal, and inhalation routes of exposure, moderately irritating to eyes, mildly irritating to skin, and not a dermal sensitizer.

For the short-term exposure proposed for this forestry application, the 30-day dietary mouse immunotoxicity study using technical Azatin 15% Technical was considered the most relevant study for toxicity end-point selection. Observed immunotoxicity in this study was considered to be the most sensitive end point in the data package. The LOAEL in this study was 112 mg/kg bw/d based on effects on cytotoxic T-lymphocyte function. A no observed adverse effect level (NOAEL) was not established for this study. The following are the main points considered in this decision:

- The anticipated exposure for mixers, loaders, and pilots will be of intermediate duration (i.e., four to six weeks) and intermittent throughout this period (e.g., four hours a day, several days per week).

- The predominant route of exposure is dermal. Inhalation is a minor route of exposure. A comparison of toxicity following dosing by oral, dermal, and inhalation routes (acute toxicity studies) did not indicate any increased route-specific systemic toxicity. Therefore, in the absence of any short-term toxicity study on the dermal or inhalation route of exposure, a toxicology study by the dietary route is considered appropriate for occupational risk assessment.

- Azatin 15% Technical and Neemix 4.5® were of low acute toxicity via the oral route, and no significant systemic toxicity was observed at a limit dose of 5000 mg/kg bw. In a short-term (90-day) dietary toxicity study in rats, the NOAEL was 161.4 and 32.1 mg/kg bw/d for males and females, respectively, based on observed altered haematological and clinical chemistry parameters. Changes in organ weights were observed at the higher dose level of 632.4 and 161.4 mg/kg bw/d for males and females, respectively; however, no histopathological correlates were observed for these organs.

- Gender sensitivity was not clearly evident in rats: the males had a more severe proliferation of the bile ducts in the portal areas of the liver; the females had an increase in liver weight and gamma glutamyl transpeptidase levels at a lower dose level than the males.

- In rats the test compounds were not mutagenic or clastogenic in vivo and was not teratogenic. However, immunotoxicity studies indicate that neem extract may have immunotoxic potential. Based on the observed suppression of cytotoxic T-lymphocyte function, the LOAEL for immunotoxicity for Azatin 15% Technical is 112 mg/kg bw/d.

- Although a decrease in adrenal and (or) ovary weights was noted in rats following a 90-day dietary exposure, no histopathological correlates were found. However, based on its endocrine mode of action in insects and the absence of a reproductive toxicity study, the potential for this compound to cause endocrine effects cannot be ruled out. No neurological signs of toxicity were observed following dietary as well as gavage exposure at the limit dose.

An additional 10-fold safety factor beyond the standard 100-fold is recommended to take into account use of a LOAEL for potential immunotoxicity and use of a Tier I data package.

3.5 Impact on human and animal health arising from exposure to Neemix 4.5®

3.5.1 Operator exposure assessment

Neemix 4.5® is an emulsifiable concentrate containing 40.4 g azadirachtin/L or 273 g total neem solids (including azadirachtin)/L. It is proposed for commercial, restricted registration for forest and woodlands management. The product would be applied once from June to early August by aerial application at a rate of 52.8 g azadirachtin/ha or 357 g total neem solids/ha.

Since Neemix 4.5® is derived from neem seeds, it may be contaminated with aflatoxins up to a maximum concentration of 24 ppb.

Neemix 4.5® would initially be used in Newfoundland, Nova Scotia, New Brunswick, and Ontario. Although in Newfoundland the degree of infestation is approximately 40 000 ha, the area that would be treated would be 4000–5000 ha. Treatment would take place over four to six weeks. On average pilots can treat 400 ha/day. Assuming the maximum application rate is used, 142.8 kg of total neem solids would be handled by mixers, loaders, and pilots in one day.

Mixer, loader, and pilot (applicator) exposure was estimated using the Pesticide Handlers Exposure Database version 1.1 (PHED 1.1). PHED is a compilation of generic mixer, loader, and applicator passive dosimetry data with associated software that facilitates the generation of scenario-specific exposure estimates. The PHED estimates meet criteria for data quality, specificity, and quantity outlined under the North American Free Trade Agreement Technical Working Group on Pesticides. Exposure was predominately dermal, with inhalation accounting for a minor component of overall exposure. Exposure estimates were based on a the assumption that dermal absorption is equivalent to oral absorption.

To estimate exposure for each use scenario, appropriate subsets of A and B grade data were created from the mixer, loader, and applicator database files of PHED. All data were normalized for each kilogram of active ingredient handled. Exposure estimates are presented on the basis of the best-fit measure of central tendency, i.e., summing the measure of central tendency for each body part that is most appropriate to the distribution of data for that body part. The exposure estimates were based on one layer of clothing and gloves in PHED, with the exception of no gloves during ground application. A protection factor of 90% for chemical-resistant coveralls to be worn during mixing and loading was incorporated into the estimates.

The following exposure estimates and margins of exposure were derived for mixers, loaders, and pilots:

	Exposure (mg/kg bw/d)[a]	Margin of exposure based on LOAEL of 112 mg/kg bw/d [b]
Mixer and loader	0.0728	1540
Pilot	0.0213	5260

NOTE: Estimates are based on mixers and loaders wearing chemical-resistant coveralls over one layer of clothing and gloves and pilots wearing one layer of clothing and no gloves.

[a] Based on a 70-kg operator and typical North American use patterns of 400 ha/day for custom mixers, loaders, and pilots. Dermal absorption was assumed to be equivalent to oral absorption.

[b] Based on mouse immunotoxicity study.

These margins of exposure are acceptable.

Potential exposure estimates to aflatoxins were also derived using PHED based on the assumption that aflatoxins have identical transfer, deposition, and penetration characteristics as the active ingredient. Aflatoxin exposure for mixers and loaders wearing the same personal protective equipment described above was 0.0056 ng/kg bw/d. This exposure is much lower than aflatoxin intake of 1–2 ng/kg bw/d in Canadian children 1–11 years old (the age group with the highest exposure potential) from the consumption of peanuts or peanut butter. This estimate is based on results from the Health Protection Branch monitoring of aflatoxin residues in nuts and nut products (1985–1987).

3.5.2 Bystanders

Bystander exposure is expected to be low, with the provincial regulatory authorities implementing procedures such as public service announcements that would further reduce exposure potential.

3.5.3 Workers

Re-entry activities are minimal in forestry and are usually mechanized. Therefore a re-entry interval is not necessary.

4.0 Residues

4.1 Residue summary

Not applicable as this product is not intended for use on food.

5.0 Fate and behaviour in the environment

5.1 Fate and behaviour in soil

5.1.1 Soil transformation

Azadirachtin hydrolyzes at environmentally relevant pH. It is photolytically unstable. Therefore, hydrolysis and phototransformation will be the principal routes of transformation in the environment. Aerobic biotransformation of azadirachtin in soil is also a route of transformation in the environment. No major transformation products were identified in the hydrolysis, phototransformation, and biotransformation of neem extract (Appendix II, Table 1).

Azadirachtin is non-persistent to slightly persistent in aerobic soil under laboratory conditions (DT_{50} 6–25 days). A terrestrial field dissipation study was not available for review.

Azadirachtin rapidly transforms in the presence of heat, moisture, air, and sunlight.

5.1.2 Mobility

A leaching study using a 60-cm column with sandy loam forest soil showed that azadirachtin was not strongly bound to the soil particles. In this study, 21% of the applied compound was found in the top 0–10 cm, 44% in the next 10–20 cm, 16% in the bottom 20–30 cm of the column, and 8% in the leachate.

5.2 Expected environmental concentration in soil

Assuming a soil bulk density of 1.5 g/cm^3, uniform distribution of the compound throughout a soil depth of 15 cm, and an application rate of 50 g a.i./ha to bare soil, the expected environmental concentration (EEC) in soil (EEC_{soil}) of azadirachtin is 0.022 mg a.i./kg.

5.3 Fate and behaviour in water

5.3.1 Aquatic transformation

Azadirachtin hydrolyzes at environmentally relevant pH. The rate of azadirachtin hydrolysis increases with an increase in alkalinity and an increase in temperature (Appendix II, Table 2).

5.3.2 Expected environmental concentrations in water

For a forestry scenario, the Tier I EEC in water (EEC_{water}) of azadirachtin from direct overspray of a body of water (15 cm deep) at the maximum recommended application rate of 50 g a.i./ha is 0.033 mg a.i./L. As a risk was indicated by the Tier I assessment, a Tier II assessment was triggered that took into account 50% interception by the forest canopy. This rate of interception was established through interdepartmental consultation with Fisheries and Oceans, Environment Canada, Natural Resources Canada (Forestry Sector) and the PMRA in 1996.

5.4 Fate and behaviour in air

The volatility of pure azadirachtin is unknown. Neemix 4.5® has a vapour pressure of 2.85×10^2 Pa, indicating that the product is highly volatile.

6.0 Effects on nontarget species

6.1 Effects on terrestrial nontarget species

6.1.1 Terrestrial organisms

Azadirachtin is practically nontoxic to the bobwhite quail on an acute and dietary basis. It is also nontoxic to the mallard duck on a dietary basis. Azadirachtin is nontoxic to the rat on an acute and dietary basis. Azadirachtin is nontoxic to honeybees (Appendix II, Table 3).

6.1.2 Aquatic organisms

The log K_{ow} value (1.9 at 25EC) indicates that azadirachtin has a negligible potential for bioconcentration or bioaccumulation in organisms. Azadirachtin is very highly toxic to fish and highly toxic to *Daphnia magna* on an acute basis (Appendix II, Table 4).

6.2 Environmental risk assessment

Risk to terrestrial and aquatic organisms from the use of azadirachtin was assessed using the margin of safety values (toxicity end point and EEC). Azadirachtin will not pose a risk to wild birds or mammals with the proposed use because it will take 50–60 days to reach the acute and dietary no observed effect concentrations (NOECs) for birds and more than three days to reach the acute NOEC for mammals. (The 50% dissipation time (DT_{50}) of azadirachtin in forestry foliage, soil, and litter ranges from 24 to 48 hours). Bees will not be at risk because the acute contact LD_{50} is equivalent to an application rate of 2.8 kg a.i./ha (Appendix II, Table 5). The Tier I aquatic risk assessment indicated that fish and daphnids might be adversely affected (margin of safety <1) (Appendix II, Tables 5 and 6); however, a more refined assessment that assumed a 50% interception by the forest

canopy (as established through the interdepartmental consultation mentioned above) indicated low risk to these organisms.

6.3 Environmental risk mitigation

The buffer zone necessary to protect sensitive aquatic species was calculated using the Agdrift model, which assumes a fine droplet size distribution, 50% interception by the canopy, 15-m maximum boom height above the canopy, and 16 km/h maximum wind speed. The end point selected was the acute NOEC for rainbow trout, which was the most sensitive aquatic species in the data provided. Although the model indicated that no buffer zone would be required, the PMRA has introduced an additional safety factor by requiring a 50-m buffer zone around aquatic resources.

7.0 Value

7.1 Effectiveness

Insect	Scientific name	Proposed application technique	Proposed rate	Proposed product
Balsam fir sawfly	*Neodiprion abietis*	Air or ground	20–50 g a.i./ha	523–1307 mL/ha

Results were submitted from two efficacy trials conducted in Newfoundland that examined aerial and ground application of Neemix 4.5® at various rates to control BFS. In summary, in 1996, Neemix 4.5® was applied aerially on first and second instar larvae at a rate of 50 g a.i./ha and significantly reduced a BFS populations by 90% while providing some foliage protection (63% whole-tree defoliation verses an average of 82% whole-tree defoliation in untreated controls) in trees containing extremely high populations of BFS (precounts of 50 larvae per branch). A below rate application of 10 g a.i. of Neemix 4.5®, applied aerially on first and second instar larvae, did not provide much reduction in BFS populations, although defoliation was reduced. In 1999, a ground application of Neemix 4.5® applied on third and fourth instar larvae at a rate of 45 g a.i./ha provided little protection of foliage or reduction in populations, possibly because of high rainfall after spraying. Neemix 4.5® applied by ground on third and fourth instar larvae at a rate of 20 g a.i./ha reduced populations slightly compared with controls and induced molting effects in BFS larvae. Sprayed trees were not defoliated any further.

Submitted efficacy data support label claims to apply between 20 and 50 g a.i./ha. However, the data do not allow for a determination of whether the lower rates are as efficacious as the higher rate of 50 g a.i./ha and do not allow for an assessment or determination of the criteria as to when to apply the high versus the low rate. The product should be applied on early instars of BFS, as 1999 spray trials conducted on third and fourth instar larvae did not appear to work as well as 1996 trials on first and second

instars. Further efficacy data would be required to confirm when the lower rate should be used and if the higher rate is necessary.

Insect	Scientific name	Proposed application technique	Proposed rate	Proposed product
Yellow-headed spruce sawfly	*Pikonema alaskensis*	Air or ground	25–50 g a.i./ha	654–1307 mL/ha

Results were submitted from two efficacy trials that examined aerial and ground application of Neemix 4.5® at various rates to control YHSS. In summary, in 1997, Neemix 4.5®, when applied aerially at 25 g a.i./ha, reduced YHSS populations by 66% and reduced tree defoliation to 9.2% compared with a trichlorfon standard applied at 500 g a.i./ha, which reduced YHSS populations by 76% and reduced tree defoliation to 9.4% (tree defoliation in the untreated blocks was 32.6 and 39.5%). In 1999, single and double applications of Neemix 4.5® by ground at a rate of 25 g a.i./ha produced minimal reductions in YHSS populations and defoliation; however, feeding was reduced in the treatment blocks. The ground applications were made on older larvae (fourth instar) and may have been too late to have a significant impact on YHSS populations.

The data support the label claims of applying between 25 and 50 g a.i./ha and would seem to indicate that the low rate of 25 g a.i./ha is as efficacious as the higher rate of 50 g azadirachtin per hectare. Further efficacy data would be required to confirm when the lower rate should be used and if the higher rate is necessary. The product should be applied on early instars of YHSS, as the 1999 spray trials conducted on later instar larvae did not appear to work as well as the 1997 trials conducted on earlier instar larvae. Only one application of Neemix 4.5® was sprayed in all trials; it is not known whether an extra application would improve the efficacy of the product.

Insect	Scientific name	Proposed application technique	Proposed rate	Proposed product
Pine false webworm	*Acantholyda erythrocephala*	Air or ground	25–50 g a.i./ha	654 –1307 mL/ha

Results were submitted from one efficacy trial conducted in Ontario that examined aerial application of Neemix 4.5® at rates of 25 and 50 g a.i./ha to control PFW. Trees sprayed with Neemix 4.5® at rates of 25 and 50 g a.i./ha had 70.4 and 67.1% dead larvae at 9 days after treatment compared with 19.9% dead larvae found in untreated controls. End-of-season whole-tree defoliation estimates of the red pines indicated defoliation of 7.6% in trees sprayed with 25 g a.i./ha, 2.7% in trees sprayed with 50 g a.i./ha, and 40% whole-tree defoliation in untreated controls. Frass collections also indicated reduced feeding, as indicated at three weeks after treatment; one week's collection of frass from 10 trees showed 1.03 g frass collected under trees treated at 50 g a.i./ha, 2.14 g frass collected under trees treated at 25 g a.i./ha, and 16.24 g of frass collected under untreated trees. The lower rate of 25 g a.i./ha appeared to provide adequate protection of red pine foliage.

However, the data indicate that populations of PFW in the block treated at 25 g a.i./ha were approximately 33% the size of the populations of PFW treated at the higher rate of treatment of 50 g a.i./ha. Although the two rates of treatment showed comparable whole-tree defoliation of red pine (less than 10%, compared with untreated controls of 40%), it is not known from the data if the lower rate would provide the same degree of protection in trees as the higher rate with larger populations of PFW.

7.2 Alternatives

For Forestry or Woodlands use, few Pest Control Products are registered for control of sawfly species. The organophosphate insecticide fenitrothion is registered for sawfly control; another organophosphate insecticide, trichlorfon, has been used for YHSS and was used for control of BFS in Newfoundland under an Emergency Registration in 1999. It should be noted that all organophosphate insecticides are currently under re-evaluation in Canada. No other biological or chemical control products are registered for use against sawfly species in Canadian forests.

8.0 Toxic substances management policy considerations

Neem extract is derived from a natural source. Neem extract does not meet the TSMP Track-1 criteria for persistence in soil, water, and sediment or for bioaccumulation. Further, TSMP Track-1 materials as identified in Appendix II of Regulatory Directive DIR99-03 *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy* are not expected to be formed or present in the product.

9.0 Overall conclusions and regulatory decision

9.1 Assessments

9.1.1 Health risk assessment

Neem Concentrate TGAI (containing 4.5% azadirachtin) poses a slight acute toxicity hazard by the inhalation route. No significant acute hazard is associated with the oral and dermal routes.

Azatin 15% Technical (15% azadirachtin) poses no significant acute hazard via oral, dermal, or inhalation routes. The end use product (Neemix 4.5®) is moderately irritating to eyes and is mildly irritating to skin.

The Tier I data package included acute, short-term teratology, mutagenicity, and immunotoxicity studies. In mammals, Neem Concentrate TGAI is not considered to be fetotoxic or teratogenic, and both Neem Concentrate TGAI and Azatin 15% Technical are not considered to be genotoxic. A short-term study conducted in rats did not illustrate any major physiological effects in the test animals at the limit dose of 1000 mg/kg bw/d. The principal target organ was the liver.

Immunotoxicity was demonstrated in an immunotoxicity study following Neem Concentrate TGAI treatment via oral gavage in female mice with effects on spleen weight in combination with effects on the PFC assay and NK function. Azatin 15% Technical via dietary exposure caused suppression of cytotoxic T-lymphocyte function with no effect on any of the other immunotoxicity test parameters. In this study, the viability of the splenocytes was not reported and it is possible that the results seen in the cytotoxic T-lymphocyte function test are associated with decreased viability of splenocytes and are not related to dosing. Further immunotoxicity testing (Tier I) should be performed for Azatin 15% Technical and Neem Concentrate TGAI for continued forestry use in subsequent years, as well as any expansion of use with potential for subchronic and chronic exposure. The results of Tier I testing will determine a need for Tier II immunotoxicity data.

An intermediate-term mouse immunotoxicity study was determined to be the most relevant for the occupational risk assessment for mixers, loaders, and pilots. The margins of exposure (1500- to >5000-fold) for this proposed forestry use of Neemix 4.5®, calculated on the basis of typical North American use patterns, are considered acceptable.

A full toxicology data package is required before any expansion of forestry use or other uses involving subchronic and chronic exposures are to be considered for this product.

9.1.2 Environmental risk assessment

Risk to terrestrial and aquatic organisms from the use of azadirachtin was assessed using the margin of safety approach (toxicity end point and EEC). Azadirachtin will not pose a risk to wild birds or mammals with the proposed use because it will take 50–60 days to reach the acute and dietary NOECs for birds and more than three days to reach the acute NOEC for mammals. (The DT_{50} of azadirachtin in forestry foliage, soil, and litter ranges from 24 to 48 hours). Bees will not be at risk because the acute contact LD_{50} is equivalent to an application rate of 2.8 kg a.i./ha. Fish and aquatic invertebrates are unlikely to be affected at the proposed application rate assuming a 50% interception by the forest canopy. A 50-metre buffer zone provides an additional margin of safety for aquatic organisms.

9.1.3 Value assessment

Adequate data were provided from the aerial efficacy trials for BFS, YHSS, and PFW to support temporary registration; however, it was not possible to determine a clear dose response of the sawfly larvae to determine lowest effective rates. Further efficacy trials would be required in order to determine optimum rates of application.

Efficacy data generated for ground applications were inadequate to allow for efficacy assessment (late instars, rainfall events) and further data are required.

The product should be applied on early instars of sawfly.

Based on the mode of action of azadirachtin and other neem by-products in the formulation, there may be other effects besides immediate population reductions. Nonlethal effects were noted by the study authors (e.g., effects on moulting, antifeedant effects); however, these effects were not quantified in the submitted studies.

9.2 Label amendments and recommendations

Primary display panel:

The label classification will be RESTRICTED only.
The signal words WARNING EYE IRRITANT should be added.
The statement KEEP OUT OF REACH OF CHILDREN should be moved to the secondary display panel under PRECAUTIONS.

Secondary display panel:

Replace the existing statement with
KEEP OUT OF REACH OF CHILDREN.

The following changes should be added to the PRECAUTIONS section of the label:

- When handling the concentrate, and during mixing, loading, clean-up, and repairs, the following personal protective equipment must be worn: chemical-resistant coveralls over long-sleeved shirt, long pants, chemical-resistant gloves, rubber boots, protective eyewear, and headgear.
- Pilots must wear a long-sleeved shirt, long pants, shoes, and socks.
- For aerial application to forests and woodlands only. (Any reference to ground application must be removed from the label.)

The following statements on the Neemix 4.5® label are required under Environmental Hazards:

- Do not apply at a boom height higher than 15 m above canopy.
- Aerial drift is increased under certain meteorological conditions. Do not apply during periods of dead calm, when winds are gusty, or when wind speed is greater than 16 km/h at the flying height.
- For the protection of nontarget habitats, overspray, or drift to sensitive habitats must be avoided. A buffer zone of 50 downwind edge of the boom and sensitive aquatic habitats such as sloughs, ponds, lakes, rivers, streams, and wetlands. Do not contaminate these habitats when cleaning and rinsing spray equipment or containers.

Directions for Use are to be enclosed in a solid black line box along with Restricted Uses and the following text added:

- NATURE OF THE RESTRICTION: This product is to be used only in the manner authorized. Contact local pesticide regulatory authorities about use permits that may be required.
- Application is to be by air only.

9.3 Regulatory decision

Azatin 15% Technical and Neemix 4.5® have been granted a temporary registration for aerial forestry use for sawflies, pursuant to Section 17 of the PCP Regulations, subject to the generation of the following studies and clarifications:

- a revised Control Product Specification Form listing the correct common names of the impurities;
- results of the analysis for the content of aflatoxins in each batch of Azatin 15% Technical produced;
- immunotoxicity testing of Neem Concentrate TGAI and Azatin15% Technical: Tier I immunotoxicity testing using currently recommended methods, followed by Tier II immunotoxicity testing if triggers are observed in Tier I;
- efficacy data for ground application; and
- efficacy trials (aerial operational trials) conducted at the rate range proposed on the label.

List of abbreviations

a.i.	active ingredient
ADI	acceptable daily intake
BFS	balsam fir sawfly
CAS	Chemical Abstracts Service
DT_{50}	dissipation time at 50%
EEC	expected environmental concentration
EPA	Environmental Protection Agency
IUPAC	International Union of Pure and Applied Chemistry
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
LOAEL	lowest observed adverse effect level
MAS	maximum average score
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
MIS	maximum irritation score
NK	natural killer cell
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
PCP	Pest Control Products
PFB	pine false webworm
PFC	plaque-forming cell assay
PHED	Pesticide Handlers Exposure Database
ppb	parts per billion
ppm	parts per million
PMRA	Pest Management Regulatory Agency
$t_{½}$	half-life
TSMP	Toxic Substances Management Policy
YHSS	yellow-headed spruce sawfly

Appendix I Toxicology

Table 1 Neem Concentrate TGAI

Study type	Species and strain and dose	LD_{50} (mg/kg bw) and LC_{50} (mg/L)	Degree of toxicity and significant effects
Acute toxicity			
Oral	Rat (Sprague-Dawley), 5/sex 5000 mg/kg bw purity: 4.5% a.i.	LD_{50} >5000 mg/kg bw	Low toxicity One animal lost hair, one animal had dark red mottled lungs.
Dermal	Rabbit (New Zealand White), 5/sex 2000 mg/kg bw purity: 4.5% a.i.	LD_{50} > 2000 mg/kg bw	Low toxicity Dermal irritation, soft stools, faecal stain, clear ocular discharge were observed.
Inhalation	Rat (Sprague-Dawley), 5 0.54 or 5.33 mg/L purity: 4.5% a.i.	LC_{50} = 0.54 –5.33 mg/L	Slight toxicity Urine stain, breathing abnormalities, swollen eyelid(s), 9 activity, rough coat, unkempt appearance, hair loss.
Eye irritation	Rabbit (New Zealand White), 2 **%**, 4 **&** 0.1 mL undiluted purity: 4.5% a.i.	Maximum average score (MAS) = 8.89 (Maximum irritation score (MIS) = 11.17 at 24 h)	Mildly irritating Corneal opacity (1/6) and conjunctivitis (6/6), resolved by day 7–10.
Dermal irritation	Rabbit (New Zealand White), 2 **%**, 4 **&** 0.5 mL undiluted purity: 4.5% a.i.	MAS = 1.04	Slightly irritating Erythema and edema resolved by 72 h.
Dermal sensitization (Buehler test)	Guinea Pig (Dunkin-Hartley), 20 **%** purity: 4.5% a.i. 40% (1st induction), 100% (2nd and 3rd inductions and challenge)	Negative	Not a dermal sensitizer

Study	Species and strain or cell type	Dose	Significant effects and comments
Genotoxicity			
Ames test	*S. typhimurium* ± S9 purity: 2.3% a.i.	100, 333, 667, 1000, 3330 or 5000 Fg/plate	Negative
Forward mutations at the thymidine kinase locus (in vitro)	Mouse lymphoma L5178Y cell line, ± S9 purity: 2.3% a.i.	12.5–150 Fg/mL	Negative
Structural chromosomal aberrations in vivo (micronucleus test)	Mice purity: 4.5% a.i.	1250, 2500 or 5000 mg/kg bw	Negative
Study	**Species (strain) and dose**	**NOAEL and LOAEL (mg/kg bw/d)**	**Significant effects at different doses (mg/kg bw/d) and comments**
Subchronic toxicity			
Dietary (90 days)	Rat (Sprague-Dawley Control: CD®BR VAF Plus), 10/sex/group 0 or 1000 mg/kg bw/d purity: 4.5% a.i.	LOAEL: 1000 NOAEL: Not determined	1000: \ body wt & body wt gain (**%**,**&**); \ MCV & MCH (**%**); \ leukocytes (**&**), \ lymphocytes (**&**), \ monocytes (**&**), [reticulocytes (**&**), \ glucose (**%**,**&**), [cholesterol (**&**), [creatinine (**%**,**&**), \ triglycerides (**%**,**&**), [alkaline phosphatase (**%**,**&**), \ organ wts (kidney, heart & adrenal in **%**,**&** and ovary in **&** with no histopathology observed); [liver wts (**%**,**&**), bile duct proliferation (**%**,**&**)
Reproductive and developmental toxicity			
Teratogenicity	Rat (Sprague-Dawley), 25/group 10, 100 or 1000 mg/kg bw/d by gavage on gestation days 6–15 purity: 4.5% a.i.	Maternal: NOAEL = 1000 Embryo or fetal: NOAEL = 1000	No toxicity was observed up to the dose level of 1000 mg/kg bw/d (high dose). Not teratogenic
Special studies (immunotoxicity)			
Gavage (30 d)	Mice ($B_6C_3F_1$), 40 **&**/dose 0, 250, 500, or 1000 mg/kg bw/d purity: 4.5% a.i. Positive controls: Cyclophosphamide (80 mg/kg bw), *N*-deacetyl-*N*-methylcolchine (0.1 Fg/mL) and recombinant human interleukin-2 (optimal concentration)	LOAEL: 250 NOAEL: Not determined	$250: \ body wt gain, \ food consumption, [water intake, \ spleen wt, \ IgM antibody forming cells in response to sheep red blood cells, \ basal NK cell activity 1000: [platelet counts, \ augmented (IL-2) NK-cell function.

Table 2 Azatin 15% Technical and Neemix 4.5®

Study type	Species, strain, and dose	LD_{50} (mg/kg bw) and LC_{50} (mg/L)	Degree of toxicity and significant effects
Acute toxicity for Azatin 15% Technical			
Oral	Rat (Sprague-Dawley) 5/sex 5000 mg/kg bw purity: not stated	LD_{50} > 5000 mg/kg bw	Low toxicity Lethargy, hunched posture.
Dermal	Rabbit (New Zealand White); 5/sex 2000 mg/kg bw purity: not stated	LD_{50} > 2000 mg/kg bw	Low toxicity Dermal irritation, transient diarrhea.
Inhalation	Rat (Sprague-Dawley) 2.41 mg/L (4 h) purity: not stated	LC_{50} > 2.41 mg/L	Low toxicity Clear nasal discharge, salivation, redness around the eyes and rales, mouth breathing, wheezing.
Eye irritation	Rabbit (New Zealand White), 3/sex 0.1 g undiluted purity: 8.65% a.i.	MAS = 2.2	Minimally irritating No corneal opacity, iritis (2/6) at 1-h only, erythema and chemosis (6/6), resolved by day 2–3.
Dermal irritation	Rabbit (New Zealand White), 3/sex 0.5 g undiluted purity: 8.6% a.i.	MAS = 0	Non-irritating
Dermal sensitization (Buehler test)	Guinea pig (Hartley), 10 **%**/group purity: 19.2% a.i. 25% (induction), 0.5% (challenge)	Negative	Not a dermal sensitizer
Acute toxicity for Neemix 4.5®			
Oral	Rat (Sprague-Dawley), 5/sex 5000 mg/kg bw	LD_{50} > 5000 mg/kg bw	Low toxicity Transient incidences of rales, urine stains, rough coat, dark material around the fecal area.
Dermal	Rabbit (New Zealand White), 5/sex 2000 mg/kg bw	LD_{50} > 2000 mg/kg bw	Low toxicity Transient incidences of faecal stain and dark material around the fecal area.
Inhalation	Rat (Sprague-Dawley), 5/sex 2.05 mg/L (4 h)	LC_{50} > 2.05 mg/L	Low toxicity Breathing abnormalities, 9 defecation, wobbly gait, 9 activity, piloerection, lacrimation, urine stain and dark material around the fecal area.
Eye irritation	Rabbit (New Zealand White), 6 **&** 0.1 mL undiluted	MAS = 23.89 (MIS = 39 @ 1h in 1 animal)	Moderately irritating Corneal opacity (4/6) at 24 h, resolved by day 10.

Study type	Species, strain, and dose	LD_{50} (mg/kg bw) and LC_{50} (mg/L)	Degree of toxicity and significant effects
Dermal irritation	Rabbit (New Zealand White), 1 **%** and 5 **&** 0.5 mL undiluted	MAS = 1.71	Mildly irritating Very slight to slight erythema (6/6), resolved by day 7.
Dermal sensitization (Buehler test)	Guinea pig (Hartley albino), 5/sex/group 25, 50, 75, or 100% (induction & challenge)	Negative	Not a dermal sensitizer
Study	**Species or strain or cell type**	**Doses employed**	**Significant effects and comments**
Genotoxicity			
Ames test	*S. typhimurium* ± S9 (purity: 8.6% a.i.)	5, 1, 0.5, 0.05, or 0.005 mg/plate	Negative
Study	**Species or strain and doses**	**NOAEL or LOAEL (mg/kg bw/d)**	**Significant effects at different doses (mg/kg bw/d) and comments**
Subchronic toxicity			
Dietary (90 d)	Rat (Sprague-Dawley Crl:CD®BR VAF Plus), 10/sex/group 0, 500, 2500 or 10 000 ppm (0, 32.1, 161.4 or 632.4 mg/kg bw/d) purity: 7.74 % a.i.	LOAEL: 632 (**%**) 161 (**&**) NOAEL: 161 (**%**) 32 (**&**)	161.4: [gamma glutamyl transpeptidase (**&**), [liver wt (**&**) 632: \ body wt, body wt gain & food consumption (**%**,**&**); \ MCV, MCH & MCHC (**%**); \ haemoglobin, hematocrit & MCV (**&**); [blood urea nitrogen (**%**), [gamma glutamyl transpeptidase (**%**,**&**), [creatinine (**&**), [liver wt (**%**,**&**) and \ ovary wt (**&**) with no histopathology observed
Special studies (immunotoxicity)			
Dietary (30 d)	Mice ($B_6C_3F_1$), 40 **&**/dose 0, 500, 1250 or 5000 ppm (0, 112, 295 or 1100 mg/kg bw/d) purity: 7.74% a.i. Positive controls: Cyclophosphamide (80 mg/kg bw), *N*-deacetyl-*N*-methylcolchine (0.1 Fg/mL) and recombinant human interleukin-2 (optimal concentration)	Immunotoxicity LOAEL: 112 NOAEL: Not determined	$112: \ cytotoxic T-lymphocyte function 1100: \ body weight gain possibly due to palatability, [platelet counts

Appendix II Environmental Assessment

Table 1 Summary of terrestrial fate and transformation data

Process	End point	Comments
Hydrolysis	$t_{½}$ at 20EC pH 4 19 d pH 7 13 d pH 10 2 h	Buffered solutions. Hydrolysis is greatly influenced by pH in the order pH 10>>pH7>pH4. Hydrolysis is a principal route of transformation at neutral and basic pH.
	$t_{½}$ at 20EC pH 8±0.5 7 d	Pond water. Hydrolysis is a route of transformation at neutral pH.
	$t_{½}$ at 35EC pH 5 11.5 d pH 7 2.4 d pH 8 0.5 d	Buffered solutions. Hydrolysis is a principal route of transformation at neutral and basic pH. At 25EC and pH 7, $t_{½}$ was 11 d; hydrolysis of azadirachtin is greatly influenced by temperature.
	$t_{½}$ at 35EC pH 6.2 21 d pH 7.3 2 d pH 8 0.5 d	Natural waters. Hydrolysis is a principal route of transformation at neutral and basic pH.
Phototransformation	$t_{½}$ 7 d	Study conducted on plant. Phototransformation is a principal route of transformation.
Aerobic biotransformation	DT_{50} 26 d at 22EC	Greenhouse study on nursery soil. Aerobic biotransformation will be a route of transformation.
	DT_{50} 6 d	Study conducted with Margosan O (0.25% azadirachtin). The study is a combination of biotransformation and leaching. As such, the methodology did not conform with guidance offered in T-1-255, Guidelines for Determining Environmental Chemistry and Fate of Pesticides.
Anaerobic biotransformation	No data available.	
Adsorption or desorption	K_{oc} 5.1–7.9	Azadirachtin has high mobility in forestry sandy loam soil.
Soil column leaching	21% in 0–10 cm 44% in 10–20 cm 16% in 20–30 cm 8% in leachate	Azadirachtin has a potential for leaching in sandy loam soil.
EEC in soil	0.022 mg a.i./kg dry soil	

Table 2 Summary of aquatic fate and transformation data

Process	End point	Comments
Hydrolysis	$t_{½}$ at 20EC pH 4 19 d pH 7 13 d pH 10 2 h	Buffered solutions. Hydrolysis is greatly influenced by pH in the order pH 10 >> pH7 > pH4. Hydrolysis is a principal route of transformation at neutral and basic pH.
	$t_{½}$ at 20EC pH 8±0.5 7 d	Pond water. Hydrolysis is a route of transformation at neutral pH.
	$t_{½}$ at 35EC pH 5 11.5 d pH 7 2.4 d pH 8 0.5 d	Buffered solutions. Hydrolysis is a principal route of transformation at neutral and basic pH. At 25EC and pH 7 $t_{½}$ was 11d; hydrolysis of azadirachtin is greatly influenced by temperature.
	$t_{½}$ at 35EC pH 6.2 21 d pH 7.3 2 d pH 8 0.5 d	Natural waters. Hydrolysis is a principal route of transformation at neutral and basic pH.
Phototransformation	No data available.	
Aerobic biotransformation	No data available.	
Anaerobic biotransformation	No data available.	
EEC in water (Tier I, direct overspray)	0.033 mg a.i./L	Forestry use

Table 3 Summary of toxicity of azadirachtin for terrestrial organisms

Group	Organism	Study	NOEC	LD_{50} and LC_{50}	Degree of toxicity
Birds	Bobwhite quail	acute oral	29.2 mg a.i./kg bw	LD_{50} > 225 mg a.i./kg bw	Moderate
	Bobwhite quail	acute oral	477 mg a.i./kg diet	LC_{50} > 477 mg a.i./kg diet	Moderate
	Bobwhite quail	dietary	1111 mg a.i./kg diet	LC_{50} > 1111 mg a.i./kg diet	Slight
	Bobwhite quail	dietary	316 mg a.i./kg diet	LC_{50} > 562 mg a.i./kg diet	Moderate
	Mallard duck	dietary	278 mg a.i./kg diet	LC_{50} > 1111 mg a.i./kg diet	Slight
Mammals	Rat	acute oral		LD_{50} > 5000 mg Azatin/kg bw	None
	Rat	acute oral		LD_{50} > 5000 mg Neemix/kg bw	None
	Rat	90 d dietary (7.74% a.i.)	LOAEL: 632 mg Azatin/kg bw/d (**%**) 161 mg Azatin/kg bw/d (**&**) NOAEL: 161 mg Azatin/kg bw/d (**%**) 32 mg Azatin/kg bw/d (**&**)		
	Mouse	30 d (7.74% a.i.)	LOAEL: 112 mg Azatin/kg bw/d(**&**)		Potentially immunotoxic
Soil organisms	Earthworm	acute		0.0264 kg a.i./ha (field application) had no effect on population	
Predators and parasites	Honeybees	acute contact		LD_{50} > 2.5 Fg a.i./bee	Moderate

Table 4 Summary of toxicity of azadirachtin to aquatic organisms

Group	Organism	Study	NOEC (mg a.i./L)	LC_{50} (mg a.i./L)	Degree of toxicity
Fish	Rainbow trout	Acute	0.016	0.048	Very high
	Bluegill sunfish	Acute	0.06	0.11	High
Invertebrates	Water flea	Acute	0.13	1.0	High
	Water flea	Acute	0.03	0.039	Very high

Table 5 Summary of risks to terrestrial organisms

Organism	Effect	Toxicity end point	EEC	Margin of safety	Risk	Mitigative measures
Bobwhite quail	Acute oral	NOEC = 29.2 mg a.i./kg bw	6 mg a.i./kg dw	60 days	no risk	not required
Bobwhite quail	Dietary	NOEC = 316 mg a.i./kg diet	6 mg a.i./kg dw	52.7	no risk	not required
Mallard duck	Dietary	NOEC = 278 mg a.i./kg bw	1.7 mg a.i./kg dw	164	no risk	not required
Rat	Acute oral	LD_{50} > 5000 mg Neemix 4.5®/kg bw (i.e., >1111 mg a.i./kg bw)	25.2 mg a.i./kg dw	>3.3 days	no risk	not required
Earthworm	Acute	0.0264 kg a.i./ha (field application) had no effect on population	0.022 mg a.i./kg		no risk	not required
Honeybees	Acute contact	LD_{50} > 2.5 Fg a.i./bee or 2.8 kg a.i./ha*	50 g a.i./ha		no risk	not required

* Fg/bee is converted to g/ha by multiplying with 1.12.

Table 6 Summary of Tier I risk assessment to aquatic organisms

Organism	Effect	NOEC (mg a.i./L)	EEC (mg a.i./L)	Margin of safety	Risk
Water flea	Acute	0.03	0.033	0.9	Risk*
Rainbow trout	Acute	0.016	0.033	0.4	Risk*

* Tier II assessment is triggered.